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Evidence for High-Risk Haplotypes and (CGG)n Expansion in Fragile X Syndrome in the Hellenic Population of Greece and Cyprus

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The expansion of the trinucleotide repeat (CGG)n in successive generations through maternal meiosis is the cause of fragile X syndrome. Analysis of CA repeat polymorphisms flanking the FMR-1 gene provides evidence of a limited number of "founder" chromosomes and predisposing high-risk haplotypes related to the mutation. To investigate the origin of mutations in the fragile X syndrome in the Hellenic populations of Greece and Cyprus, we studied the alleles and haplotypes at DXS548 and FRAXAC2 loci of 16 independent fragile X and 70 normal control chromosomes. In addition, we studied 191 unrelated normal X chromosomes for the distribution and frequencies of CGG alleles. At DXS548, 6 alleles were found, 2 (194 and 196) of which were represented on fragile X chromosomes. At FRAXAC2, 6 alleles were found, 4 of which were present on fragile X chromosomes. Sixteen haplotypes were identified, but only 5 were present on fragile X chromosomes. The highest number of CGG repeats (≥ 33) were associated with haplotypes 194-147, 194-151, 194-153, and 204-155. The data provide evidence for founder chromosomes and high-risk haplotypes in the Hellenic population. © 1996 Wiley-Liss, Inc.

KEY WORDS: fragile X syndrome, haplotype, CGG repeats, hellenic population

INTRODUCTION

Fragile X (fraX) syndrome is the most common form of inherited mental retardation and is caused by an expansion of the unstable (CGG)n sequence in the 5′ UTR exon of the FMR-1 gene [Ashley et al., 1993a].

Although not expected for a relatively common X-linked disease, full mutations seem to occur in a number of "predisposed" chromosomes. Data of high-risk haplotypes or linkage disequilibrium between the fraX mutation and closely linked CA repeats have been reported in various Caucasian populations [Richards et al., 1992; Buyle et al., 1993; Oudet et al., 1993a,b; Macpherson et al., 1994; Malmgren et al., 1994]. High-risk haplotypes have been associated with intermediate or borderline number of CGG repeats, suggesting that these haplotypes may be prone to transition from the normal to the abnormal range of repeats [Kunst and Warren, 1994; Montagnon et al., 1994]. Most studies have implemented haplotypes of flanking microsatellite markers DXS548 and FRAXAC2 (located 150 kb and 10 kb, respectively, from the CGG repeat) to show possible susceptibility of certain allelic combinations in the development of fraX mutations [Richards et al., 1991; Riggins et al., 1992].

We used microsatellite markers DXS548 and FRAXAC2 to search for linkage disequilibrium in normal individuals and fraX patients of Hellenic origin in Greece and Cyprus. These markers show no recombination with FRAXA or with one another. In addition, we studied the number of CGG repeats to correlate the expansions with the haplotypes in this population.

MATERIALS AND METHODS

Typing of CA repeats DXS548 and FRAXAC2 was performed on 16 unrelated fraX patients and 70 normal control individuals from different parts of Greece and Cyprus. The diagnosis of fraX syndrome was based on clinical criteria, standard cytogenetic procedures [Sutherland, 1979], DNA analysis with probe StB12.3 [Oberlé et al., 1991], and the number of CGG repeats [Brown et al., 1993]. Polymerase chain reaction (PCR) of CA polymorphisms was performed with oligonucleotide primers described by Verkerk et al. [1991] for DXS548 and by Richards et al. [1992] for the FRAXAC2 loci after end labeling of one primer with ^{32}P. PCR products were diluted in formamide loading buffer, loaded

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on a 6% polyacrylamide gel, and electrophoresed with molecular weight marker. Autoradiograms were read after 4–20 hr of exposure. The chi-square test was used to calculate statistical significance.

CGG repeats were amplified by nonradioactive PCR, as described by Brown et al. [1993], in 191 normal independent chromosomes from the general population, including those studied for DXS548 and FRAXAC2 haplotypes. The number of CGG repeats were calculated with three different markers: 1 kb DNA ladder (BRL), ϕX174/HaeIII (NEB), and pUCBM21DNA/HpaII-DraI-HindIII triple digest. The number of repeats was correlated to the haplotypes to identify a possible association with high or low CGG repeat number.

RESULTS

The DXS548 and FRAXAC2 microsatellite markers flanking the fraX locus were analyzed in 16 unrelated pairs of carrier mothers and affected boys to determine their haplotypes. Data on 70 normal chromosomes were provided by normal control individuals from the same population. On normal and fraX chromosomes, 6 different alleles at the DXS548 locus and 6 different alleles at the FRAXAC2 locus were observed (Table I). The most frequent alleles were 194 for DXS548 and 151 and 153 for FRAXAC2. Percentages of the most frequent alleles were similar in normal and fraX chromosomes: 80% and 87%, respectively, for 194, 30%, 50%, and 25% for 151, and 43% for 153. Alleles 196 at DXS548 and 147 and 155 at FRAXAC2 were significantly more frequent in fraX than in normal chromosomes (196: 12% vs. 4%, 147: 12% vs. 4%, 155: 19% vs. 10%). Differences in the frequencies between the 2 groups were not statistically significant, with the level of significance set at $P \leq 0.05$.

The distribution of alleles differed between normal and fraX chromosomes. At DXS548, only alleles 194 and 196 were present on fraX chromosomes, whereas all other alleles (192, 194, 196, 200, 204, 206) were found on normal chromosomes. At FRAXAC2, 4 alleles (147, 151, 153, 155) were observed on fraX chromosomes and 6 (147, 149, 151, 153, 155, 157) were observed on normal chromosomes.

Sixteen haplotypes were identified on normal chromosomes, whereas only 5 were identified on the fraX chromosomes (Table I). Haplotypes 194-151 and 194-153 were equally common in the 2 groups. Haplotype 194-147 was found frequently, and haplotype 196-155 was only found on fraX chromosomes.

Analysis of the CGG repeats revealed 29 alleles; the most frequent was 29 (41%), followed by 28 (20%) and 30 (7%), and the distribution spanned from 12 to 50 repeats. Secondary peaks were found on 20, 21, and 36 repeats. Figure 1 summarizes the findings on CGG repeats and their distribution in the Hellenic population. Forty percent of the 47 normal females tested were homozygous or had alleles different by 1 repeat. The most common CGG alleles were 28:28 and 29:29 in homozygous females and 28:29 in heterozygous females (Table II, Figs. 2, 3). We did not observe any difference in the number of CGG repeats between males and females. The highest number of repeats (≥33) identified in 12 normal individuals was associated with haplotypes 194-147 (36 repeats), 194-151 (33, 36, 40, 49 repeats), 194-153 (36, 37, 40, 41, 50 repeats), and 204-155 (43, 49 repeats) (Table I).

TABLE I. Distribution (%) of Alleles at Loci DXS548 and FRAXAC2 and Haplotypes in Fragile X and Normal Chromosomes

	No. of chromosomes (frequency of alleles)		
	FraX	Normal	Normal (≥33 repeats)
Alleles (bp)			
DXS548			
192	0	3 (0.04)	
194	14 (0.87)	56 (0.80)	
196	2 (0.12)	3 (0.04)	
200	0	1 (0.01)	
204	0	6 (0.09)	
206	0	1 (0.01)	
Total	16	70	
FRAXAC2			
147	2 (0.12)	3 (0.04)	
149	0	2 (0.03)	
151	4 (0.25)	21 (0.30)	
153	7 (0.43)	35 (0.50)	
155	3 (0.19)	7 (0.10)	
157	0	2 (0.03)	
Total	16	70	
Haplotype			
192-151	0	1 (0.01)	
192-153	0	2 (0.03)	
194-147	2 (0.12)	3 (0.04)	1 (0.08)
194-149	0	2 (0.03)	
194-151	4 (0.25)	17 (0.24)	4 (0.33)
194-153	7 (0.44)	29 (0.41)	5 (0.42)
194-155	1 (0.06)	3 (0.04)	
194-157	0	2 (0.03)	
196-151	0	2 (0.03)	
196-153	0	1 (0.01)	
196-155	2 (0.12)	0	
200-155	0	1 (0.01)	
204-151	0	1 (0.01)	
204-153	0	3 (0.04)	
204-155	0	2 (0.02)	2 (0.17)
206-155	0	1 (0.01)	
Total	16	70	12

DISCUSSION

The genetic history of the population in Greece does not favor founder effects because the country is located at the crossroads of Europe and Asia. Other hereditary diseases, such as β-thalassemia and cystic fibrosis, exhibit a wide spectrum of mutations or a comparatively lower frequency of common mutations than in other European countries [Kattamis et al., 1990; Kanavakis et al., 1995].

The mutation rate for fraX syndrome has been estimated as 2.5×10^{-4} [Morton and Macpherson, 1992], and mutations are likely to appear on different risk haplotypes in different populations. Our results show that fraX mutation is not associated with a major haplotype at DXS548 and FRAXAC2 loci. However, we

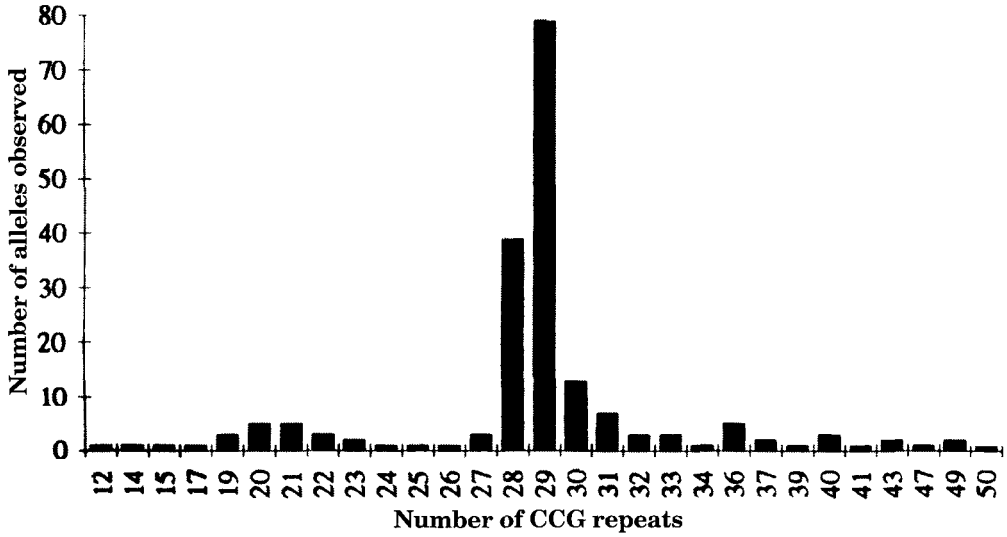

Fig. 1. Distribution of CGG repeats in the Hellenic population in Greece and Cyprus.

have identified alleles and haplotypes that either present on only fraX chromosomes or have a higher, but not significantly higher, frequency than the haplotypes found on the normal chromosomes. In accordance with the haplotypes, only a fraction of alleles, 2 in DXS548 and 4 in FRAXAC2, were present in the mutated chromosomes. Not all allele combinations were found in the fraX haplotypes.

TABLE II. Number of CGG Repeats in Females and Frequency of Heterozygous and Homozygous Alleles

ΔCGG	No. of females	%
Homozygous		
32:32	1	0.0217
30:30	1	0.0217
29:29	11	0.2391
28:28	4	0.0870
20:20	1	0.0217
19:19	1	0.0217
Heterozygous		
18:29	1	0.0217
29:50	1	0.0217
37:40	1	0.0217
31:36	1	0.0217
30:49	1	0.0217
21:30	1	0.0217
21:28	2	0.0435
21:29	1	0.0217
28:29	6	0.1304
30:33	1	0.0217
30:29	1	0.0217
28:36	1	0.0217
29:36	1	0.0217
21:26	1	0.0217
31:37	1	0.0217
19:28	1	0.0217
22:28	1	0.0217
29:43	1	0.0217
29:41	1	0.0217
24:28	1	0.0217
32:49	1	0.0217
27:40	1	0.0217

Our data show that the spectrum of haplotypes in the Hellenic population is wider, as expected, than that identified in Scandinavian populations [Oudet et al., 1993b; Malmgren et al., 1994].

Haplotype 196-155 is found only on fraX chromosomes, and haplotype 194-147 has a frequency threefold higher than on normal chromosomes. The other 3 fraX haplotypes (Table I) have on average the same frequency as normal haplotypes and also have comparable frequencies in other European populations [Oudet et al., 1993a]. This finding indicates that primary mutation events happen on a limited number of chromosomes from which most contemporary Hellenic mutations may have originated.

The distribution of CGG repeat numbers in the Hellenic population was comparable with other populations, but the highest frequencies were clustered in the 29 and 28 repeats (Fig. 1), suggesting that certain alleles are more stable than others [Fu et al., 1991; Brown et al., 1993; Mila et al., 1994]. The fact that the highest number of repeats is associated with 3 of the 5 fraX haplotypes in this population (194-147, 194-151, 194-153) may demonstrate that chromosomes bearing these haplotypes have high risk for expansion, especially haplotype 194-147, which was coincided with more than 33 repeats. Haplotype 204-155, although not represented in this patient population, has been described as the major high-risk haplotype in France [Montagnon et al., 1994].

In conclusion, high-risk haplotypes exist in the Hellenic population for the fraX syndrome, facilitatating selection and making the DXS548–FRAXAC2 microsatellite markers informative for the indirect diagnosis of fraX families.

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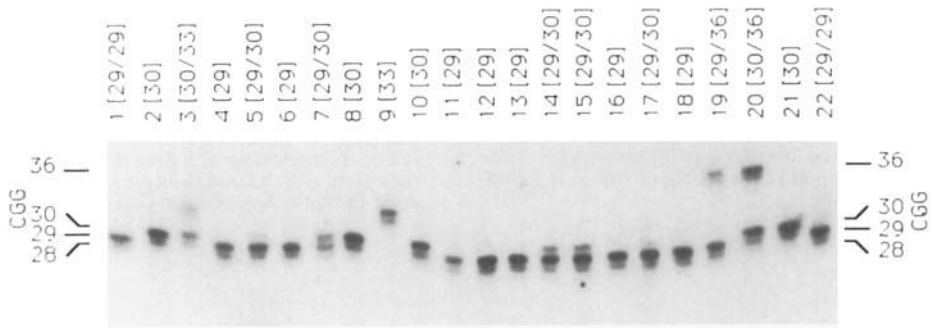

Fig. 2. CGG repeats observed in clinically normal individuals; lanes 1 and 2 served as controls. The number of CGG repeats ranged from 17 to 85.

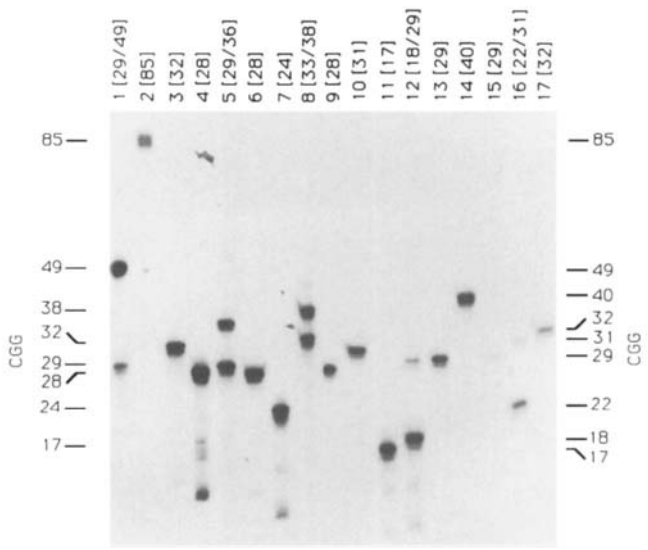

Fig. 3. Common CGG repeats observed among normal individuals. Lanes 1, 3, 5, 7, 14, 15, 17, 19, 20, and 22 are from females who show homozygosity and heterozygosity by 1 or more alleles. CGG repeat sizes were calculated with three different markers: 1 kb DNA ladder (BRL), ϕX174 HaeIII (NEB), and pUCBM21DNA HpaII-Dra1-HindIII.

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